IMPLEMENTATION OF HYDROGEN GENERATOR FOR BIOPHARMACEUTICAL INDUSTRIES.

by

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A thesis submitted to Johns Hopkins University in conformity with the requirements for

the Masters degree

Baltimore, Maryland

May 2021

Abstract

Laboratory managers are faced with many challenges to effectively and safely run a laboratory while producing good results from their analysis as well as not comprising on safety of personnel and to building. Hydrogen gas is an important gas in many laboratory applications and while its use is essential, it poses some hazards especially when stored under pressurized gas cylinders. The focus of this work is to consider an alternative for the generation of hydrogen using a hydrogen gas generator, the validation/commissioning of the hydrogen gas generator and the possible factors to consider when switching from hydrogen cylinders to hydrogen gas generators. Benefits of using the hydrogen gas generator are also a focus. The study covers what a manager needs to do to fully commission a hydrogen gas generator in a good manufacturing practice (GMP) area and develop a Standard Operating Procedure (SOP) for the operation of the generator.

Primary Reader and Advisor: Dr. Chao Wang

Secondary Reader: Dr. Marc Donohue

Acknowledgments

I am very grateful to my advisor, Professor Chao Wang, for his guidance along the road of my Masters study. Prof. Chao Wang profound insights and broad vision in research have been a role model to me. I am also thankful to him for always being patient and for giving me the freedom to explore different ideas such as the Co-op track as well as conduct research in his laboratory, which not only helped me to shape my independent research interests but also made the research process enjoyable. I am also thankful to Eng. Cameron Waller of GSK for giving me the opportunity to do my Co-op program and for his unwavering support throughout my time with him. I would also like to express my gratitude to Dr. Donohue for serving as my second reader and Dr. Sakul for organising the MSE Day.

Dedication

This thesis is dedicated to my late Mother, Mary Nyakio, for her eternal love, trust, support, and prayers.

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Chapter 1

Introduction

Hydrogen, for GC applications, can be generated at minimal pressures in a laboratory or any facility to provide significant safety and convenience compared to the use of gas from cylinders. When gas chromatography (GC) is used to separate mixtures, the selection of the suitable carrier gas, fuel gas and their optimum source are critical decisions for the laboratory manager or the principal investigator (PI) (Connor, 2015). The manager needs to select the carrier gas and fuel gas that will provide the desired output or separation in the practical minimum period of time to optimize the production of the laboratory. Furthermore, once the suitable gas has been identified, the manager should then assess the various possible sources of that gas to decide how the gas should be supplied to ensure safety, compliance with Environmental, Health and Safety (EHS) recommendation, convenience, and minimize the cost of the gas in the long run.

In the past, nitrogen and helium has been used as the carrier gas in Gas Chromatography applications (Connor, 2015). Nitrogen though abundantly present in the atmosphere has several disadvantages such as long analysis time due to high viscosity resulting in low velocity and not producing the best results. Helium produces good analysis results but has the downside of not being renewable, making it so expensive. Helium also takes relatively more time to produce results due to its high viscosity compared to hydrogen. In recent decades, research has been undergoing on the use of hydrogen gas as a carrier gas and has shown significant results in reduction of the time of analysis due to increased speed of flow of the hydrogen gas as a result of low viscosity of hydrogen. Hydrogen use is renewable making its use relatively cheap compared to helium (Bartram & Froehlich, 2010). In most cases when hydrogen gas is used, it is normally supplied to the application instrument from a pressurized gas cylinder with proper pressure reduction valves and piping. While this approach is fairly-straight forward, it suffers from a number of disadvantages. Some of these are listed below:

a) Poses dangers in working with pressurized gas. These are several hazards which include ergonomic hazards when moving the cylinders due to their heavy weight. An undetected leak from a high-pressure cylinder could pose additional hazards such as asphyxiation risk to personnel and explosion risk in case of a naked flame present.

b) A cylinder can act as a missile when the valve is accidentally knocked out forcing a large amount of gas to escape in a small opening.
c) Cost of the cylinders and one is at the mercy of the cylinder owners, and
d) The inconvenience of having to replace cylinders from time to time even during extreme weather conditions.

A compressed gas is defined by the Department of Transportation (DOT) as "any material or mixture which exerts in the packaging an absolute pressure of 280 kPa (40.6 psia) or greater at 20°C (68°F)." Handling compressed gases is considered more hazardous than handling solids or liquids. This is because of the high pressure, flammability of some gases and most gases are odorless thus a leak can go undetected. Improper pressure regulations at the valves could damage applications instruments. (Compressed Gas Association, Inc., 1985)

Knowledge of the gas to be handled.

It is crucial that the user be familiar with the hazardous properties of a compressed gas such as flammability, chemical activity, toxic nature among others. It is at times difficult to determine a major hazard of a particular gas as this would be mainly determined by its use. In a laboratory in presence of a naked flame, explosion is likely to be the major hazard when hydrogen gas is used. In the same laboratory without the naked flame and without a gas detection system, asphyxiation would likely be the major hazard when hydrogen is used especially because it is odorless. When hydrogen is used in GC applications in a laboratory that uses Flame Ionization Detectors, an explosion hazard is likely to be the major hazard due to the presence of a flame.

Table 1: Flammability of common flammable gases in air

Gas	Lower Explosive Limit	Higher Explosive Limit
Methane	5.30%	15%
Propylene	20%	10.50%
Propane	2.20%	9.50%
Hydrogen	4%	74%
Acetylene	2.20%	85%

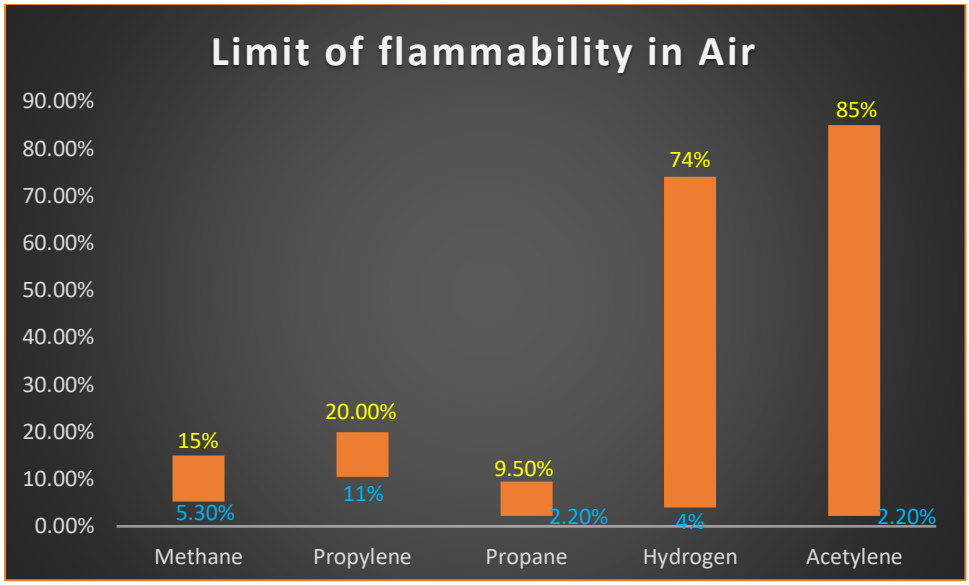


Figure 1: Flammability of common flammable gases in air showing lower (blue font) and upper (yellow font) explosive limits.

The most flammable gas is acetylene which readily combusts when mixed with air. This is followed by hydrogen gas which has a lower explosive limit of 4% and a higher explosive limit of 74%. This is a very wide combustion limit which increases the risks of using large amounts of hydrogen in a facility and can easily be compared in the bar chart above with some common gases such as methane and propylene that are common domestic use gases for cooking.

Hydrogen gas as a carrier gas for Gas Chromatography (GC)

Hydrogen gas is a very important carrier gas for gas chromatography applications and gives a list of significant benefits in comparison to the use of other commonly used carrier gases such nitrogen and helium. The main benefit of hydrogen gas is the fact that it can lead to a drastic reduction of the required time for a given separation (Connor, 2015). Because the use of hydrogen gas provides a remarkable reduction in the separation time due to the increased velocity leading to increased flow rate caused by reduction in hydrogen viscosity, the laboratory analyst could reduce the column temperature for separation. Reducing the analysis temperature has an overall effect of increasing the lifetime of the analysis column which leads to further economic benefit.

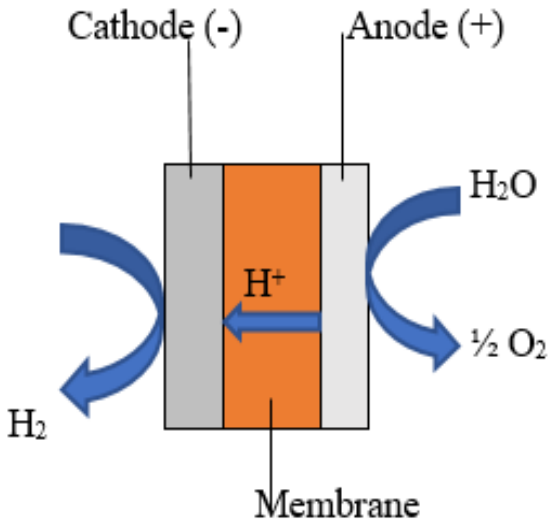
In addition to its use as a carrier gas, hydrogen gas is also utilized in Gas Chromatography (GC) as a fuel gas for flame-ionization detectors (FIDs).

Although both hydrogen and helium have more satisfactory results than nitrogen, there are several drawbacks to the use of helium gas. Helium gas is a non-renewable resource with limited availability in many pars of the world making it a very expensive gas to use and its cost continues to rise year in year out (Connor, 2015). On the contrary, hydrogen gas is readily available via the electrolysis of water which is gaining popularity or as a high-pressure gas in hydrogen gas cylinders.

Hydrogen gas generators

Hydrogen gas generators are compact stand-alone units capable of delivering hydrogen gas at low pressures and on demand. A hydrogen gas generator is truly an investment in safety, as only small volumes of gas at a very low pressure can be stored at any given time. Hydrogen gas generators make use of a Proton Exchange Membrane (PEM) to generate hydrogen gas on demand. The Proton Exchange Membrane only permits only hydrogen ions (H^+) to cross through blocking other ions. This ensures that the hydrogen gas generated at the cathode does not mix with the oxygen gas generated at the anode which would otherwise affect the purity of the two gases produced. This technology generates the highest purity of hydrogen gas. The generator monitors the pressure of the generated hydrogen gas and in case of any sudden change of pressure, this is interpreted as a leak. If a hydrogen leak is detected by the generator, a built-in sensor shuts the generation of hydrogen gas and an explosion protection system ensures the highest level of operator safety.

Figure 2:Schematic diagram of hydrogen cell generator



Generation of hydrogen via the electrolysis of water.

The hydrogen gas generator produces hydrogen gas in the laboratory through the electrolytic dissociation of water that breaks down water into its two components; hydrogen at the cathode and oxygen at the anode. This is a very safe, convenient, reliable and economical method to supply hydrogen gas for Gas Chromatography (GC) application. The electrolysis process occurs through a two-step process described below using the two equations.

Step I: $H_2O(l) \rightarrow 2\ H^+(aq) + 0.5\ O + 2e^-$ (Anode)

Step II: $2\ H^+(aq) + 2e^- \rightarrow H_2(g)$ (Cathode)

Step I: Water is broken down to form two hydrogen ions (proton), oxygen atom and two elctrons.

Step II: The two hydrogen ions formed in step I cross the membrane (PEM) to reach the cathode where they are reduced by the two electrons that were generated at the anode to form molecular hydrogen gas.

A schematic diagram of a hydrogen cell in a hydrogen gas generator is shown in Figure 2 above. The cell is the primary location or point where electrolysis takes place. The cell has a solid proton exchange membrane (PEM) that supports electrolysis. Depending on the desired flow rate and pressure of the gas, the unit operates at a potential difference of about 7V. In some generator designs a specially designed palladium membrane is added to optimize the purity of hydrogen. This palladium membrane is heated to more than 600°C which only permits hydrogen ions and its isotopes to pass through the pores. This generates hydrogen of highest purity with an oxygen content less than 0.01ppm and a water vapor content less than 1.0ppm at a pressure of 100psi. The water vapor (moisture) content can further be reduced by the use of a moisture trap that can be installed along the hydrogen line. The moisture trap is designed to remove moisture that further increases the purity of hydrogen gas generated. The use of moisture trap also protects the application instrument (GC) which might be damaged by presence of moisture and may affect analysis. (Zhifei Y., 2020)

Figure 3: Moisture trap along the hydrogen line to capture any water vapor.

Chapter 2

Methods

Overview of the equipment

The hydrogen gas generator is a bench top equipment measuring about 18in x 13.5in x 17.2in (Height x width x depth) and weighing about 50lbs. It uses deionized water to generate hydrogen gas at low pressure.

Figure 4: Schematic diagram of a hydrogen gas generator

Receiving and Inspecting the Equipment

On receipt of the equipment, we carefully inspected the packaging for any damage. We also examined all materials to ensure that all parts were shipped as listed in the shipment packaging.

Storage

Since the equipment was to be stored prior to installation for several weeks to allow good planning, it was not removed from the original packaging. We also ensured that it was stored in an upright position as indicated by the arrows on the packaging.

The storage area was secure, and the environmental conditions fell within those specified in the technical specification, that is, room temperature and humidity between 30-70%.

Unpacking

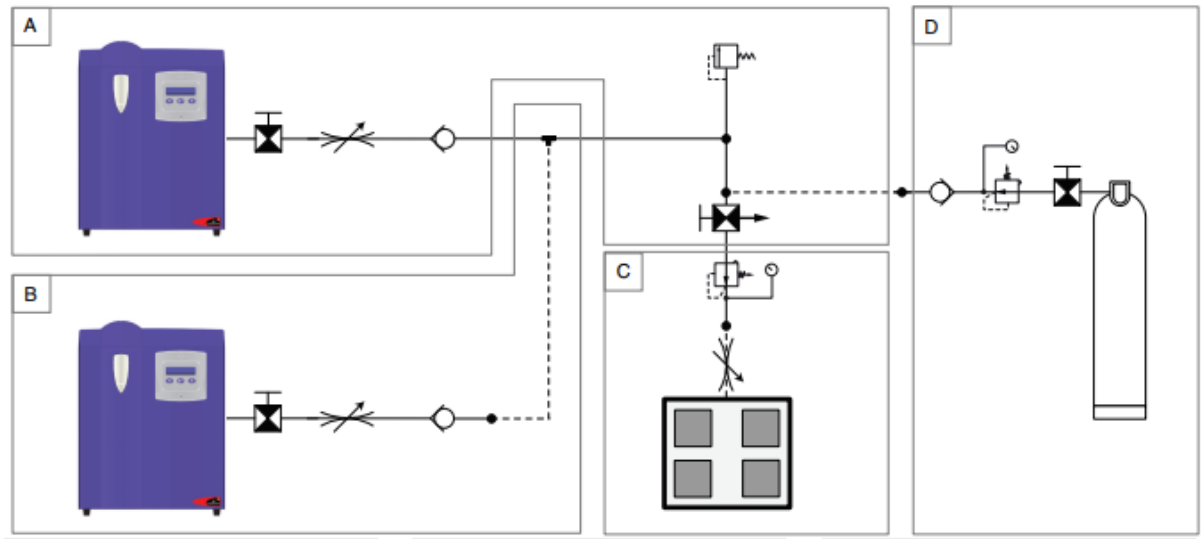
After planning and once ready to install, the equipment was removed from the packaging and signs of damage thoroughly checked. Items listed on the packaging list were also thoroughly checked.

Clearances from walls and any other barrier

A minimum clearance of 150mm (about 6in) was provided on all sides of the generator during installation. This is for air flow and to easily perform tasks to the generator such as turning it on and off, maintenance and operation. A vertical clearance of more than a metre was also provided to allow the front upper service panel to be removed during water filling. The spacing provided will also allow unrestricted access to the generator during servicing and maintenance in the future.

Installation

Figure 5: Recommended system layout by Perkin Elmer.



A	Single generator	C	Application Instrument		Non return valve
B	Multiple generators	D	Back up supply		Flow Controller
	Isolation Valve		Pressure regulator		Pressure relief valve
	3-way ball valve with vent line.				

We installed a single generator and thus followed the recommended arrangement A. Along the line we installed a moisture trap to further increase the purity of hydrogen gas by trapping any moisture before going to the GC. This also will help in protecting the application instrument (GC) that might be destroyed by moisture and could also result in unwanted peaks.

Figure 6: Installation of equipment showing the hydrogen line and moisture trap in place.

It is not possible to measure or determine the purity of the hydrogen gas generated in the laboratory. This posed a question of how we will then validate or commission the generator. We carried out a system suitability test whereby we ran samples in the Gas Chromatography which is a validated system using the hydrogen gas cylinder and then use the hydrogen gas

generator and compared the results. The following performance qualification test was drafted and a total of 6 samples were run to do the analysis.

Table 2: Performance verification

PERFORMANCE VERIFICATION				
Configuration Instruction	**Expected Result**	**Actual Result**	**Pass/ Fail**	**Date**
Perform GC cholesterol test on a previously released raw material sample using Hydrogen tank.	Cholesterol system suitability result- Passes Cholesterol Raw material MN10962 or MN11130: Assay result= 90.0%-110.0%	**System Suitability:** **Assay:**		
Perform GC cholesterol assay test on a previously released raw material sample using Hydrogen generator.	Cholesterol system suitability result- Passes Cholesterol Raw material MN10962 or MN11130: result= 90.0%-110.0%	**System Suitability:** **Assay:**		

PERFORMANCE VERIFICATION				
Configuration Instruction	**Expected Result**	**Actual Result**	**Pass/ Fail**	**Date**

Standard preparation

Cholesterol stock solution preparation at 1.0mg/mL:

Cholesterol measured weight: (100.0mg + or – 0.2mg) = 100.2mg

Volume of cyclohexane in volumetric flask: 100mL

The cholesterol standard 50 µg/mL was prepared by adding 500 µL of the 1.0mg/mL Cholesterol Stock Solution into a 10 mL volumetric flask. The solution was diluted to volume using cyclohexane. The standard was injected 6 times before the sample, and once after the samples. Two cyclohexane blank injections were performed at the beginning of the sample sequence.

Injection volumes of 1 µL.

Chapter 3

Results and Discussion

System suitability test

Table 3: System suitability test

System Suitability	Criteria	Pass/Fail
Cyclohexane blank Injections	No Interfering peak within the retention time window of the cholesterol	Pass
Cholesterol Standard 50 µg/mL		
Peak Area Response of 6 injections of the standard run before the samples	% CV	Fail
% Recovery of the Concentration of the standard run after the samples	95-105%	Fail

Table 4:Sigma Cholesterol Standard 50 µg/mL (6 injections)

Injection Number	Retention Time (min)	Peak Area
1	8.304	64.26137
2	8.304	68.00178
3	8.304	67.33208

4	8.303	68.72764
5	8.303	69.34881
6	8.303	72.26811
Average	8.304	68.32330
SD	0.001	2.621521
%CV	0.0%	3.836936%

%CV = SD/Average * 100

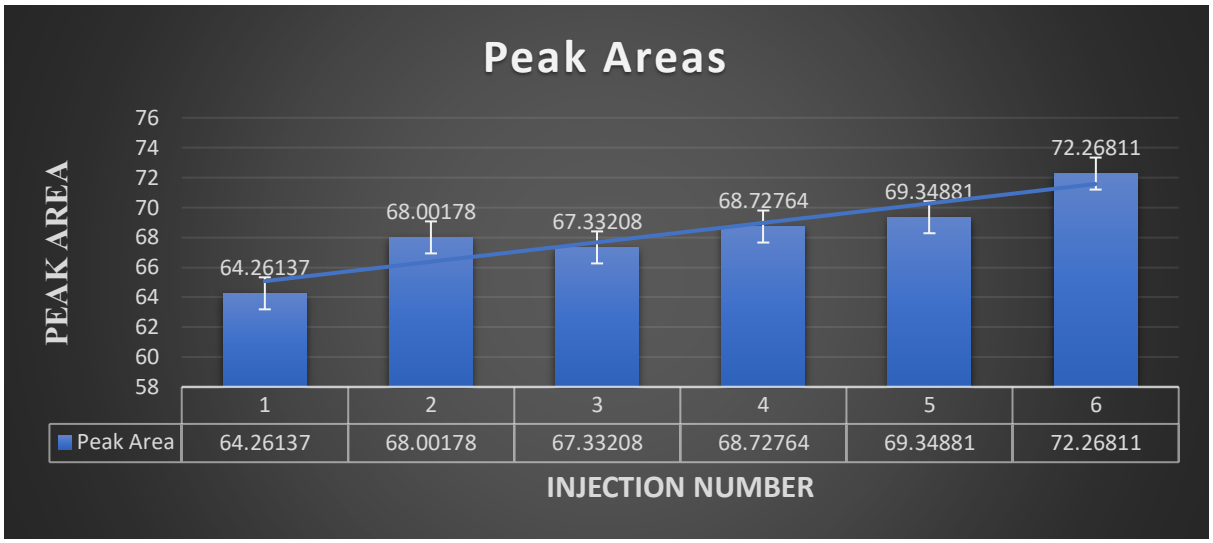


Figure 7: Bar graphs showing peak areas.

The system suitability test is used in the analytical laboratory to ensure that complete analytical system is suitable for the desired use. To commission the hydrogen gas generator, a system suitability test was done and the peak areas from the cholesterol standard runs recorded. Since this is the same sample, we would expect same peak areas to show up but it was not the case. The peak areas were different as shown in the table and bar graphs above and thus the system suitability test failed. We would carry more runs to ascertain that the problem could have been the samples or the analyst and the future plan on this work is to use a freshly prepared sample and use a different analyst to run the samples.

Conclusion

The utilization of hydrogen gas as a carrier gas for Gas Chromatography (GC) provides more quick separations than when nitrogen and helium are used. The use of hydrogen gas generators to provide hydrogen gas to application instruments in the laboratory is a viable consideration mainly for safety reasons. A hydrogen generator produces a steady stream of hydrogen gas at minimal pressure and stores a very small amount of the actual gas, therefore increasing the safety by reducing the potential of an explosion. Furthermore, the hydrogen gas generator is more convenient than gas cylinders, requires essentially minimal maintenance, and reduces the cost of hydrogen in the long run relative to the use of hydrogen gas cylinders. A single hydrogen generator can provide carrier gas for several Gas Chromatography instruments as well as the gas needed as a fuel gas. Hydrogen gas generator has built in safety features in case of a leakage, shutting down the generation of hydrogen gas, hence removing the danger of the lower explosive limit (LEL) being reached (4%). The use of hydrogen gas generator is a viable and a safe alternative over and above the use of hydrogen gas cylinders.

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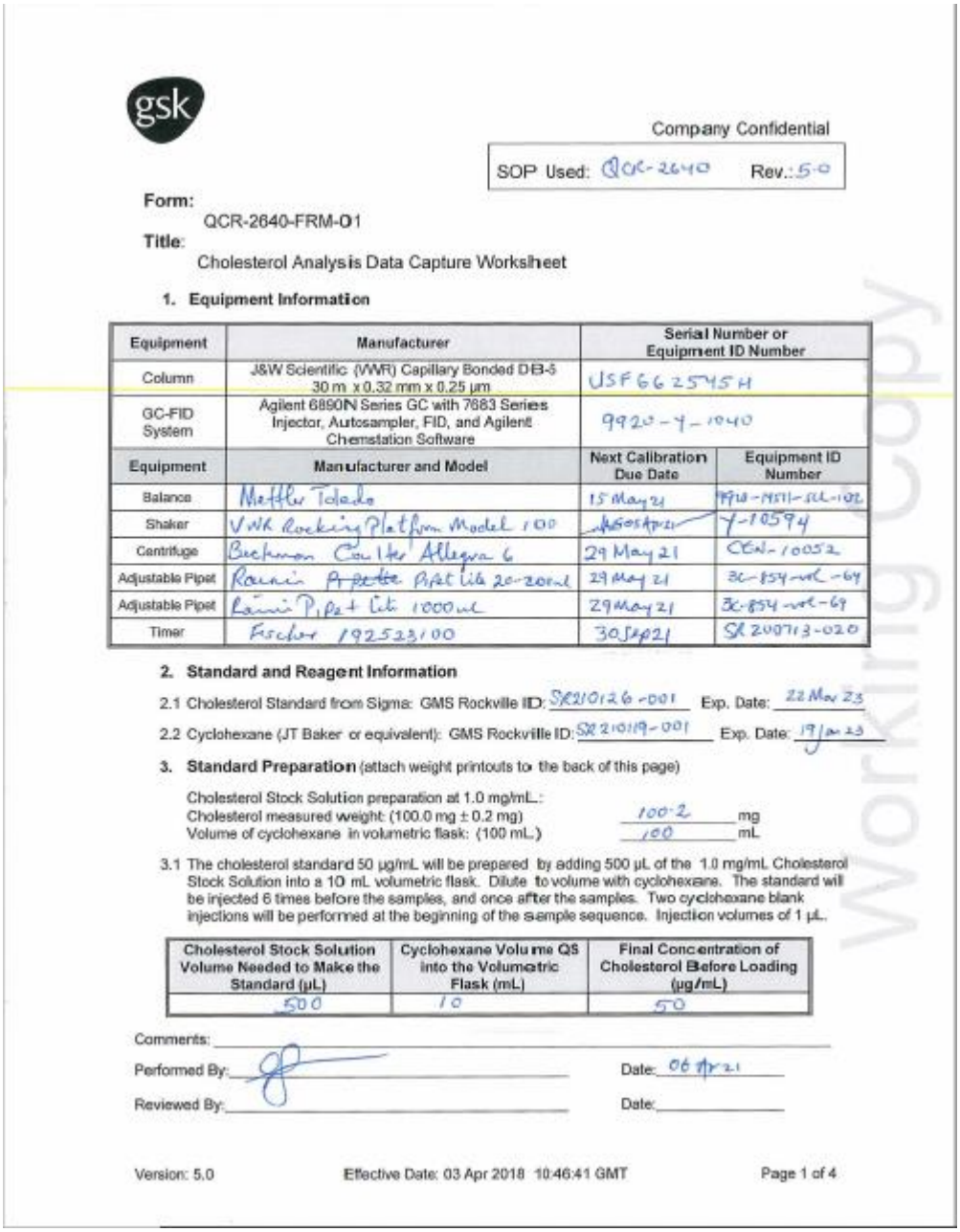
Appendices



gsk

Company Confidential

SOP Used: QCR-2640 Rev.: 5.0

Form:
QCR-2640-FRM-01

Title:
Cholesterol Analysis Data Capture Worksheet

1. Equipment Information

Equipment	Manufacturer	Serial Number or Equipment ID Number	
Column	J&W Scientific (WWR) Capillary Bonded DB-5 30 m x 0.32 mm x 0.25 µm	USF662545H	
GC-FID System	Agilent 6890N Series GC with 7683 Series Injector, Autosampler, FID, and Agilent Chemstation Software	9920-4-1040	
Equipment	**Manufacturer and Model**	**Next Calibration Due Date**	**Equipment ID Number**
Balance	Mettler Toledo	15 May 21	9918-9511-SCL-102
Shaker	VWR Rocking Platform Model 100	[illegible]	Y-10594
Centrifuge	Beckman Coulter Allegra 6	29 May 21	CEN-10052
Adjustable Pipet	Rainin Pipet lite 20-200uL	29 May 21	3C-854-uL-64
Adjustable Pipet	Rainin Pipet Lite 1000uL	29 May 21	3C-854-uL-69
Timer	Fischer 19252B/100	30 Sep 21	SR 200713-020

2. Standard and Reagent Information

2.1 Cholesterol Standard from Sigma: GMS Rockville ID: SR210126-001 Exp. Date: 22 Mar 23

2.2 Cyclohexane (JT Baker or equivalent): GMS Rockville ID: SR210119-001 Exp. Date: 19 Jan 23

3. Standard Preparation (attach weight printouts to the back of this page)

Cholesterol Stock Solution preparation at 1.0 mg/mL:
Cholesterol measured weight: (100.0 mg ± 0.2 mg) 100.2 mg
Volume of cyclohexane in volumetric flask: (100 mL) 100 mL

3.1 The cholesterol standard 50 µg/mL will be prepared by adding 500 µL of the 1.0 mg/mL Cholesterol Stock Solution into a 10 mL volumetric flask. Dilute to volume with cyclohexane. The standard will be injected 6 times before the samples, and once after the samples. Two cyclohexane blank injections will be performed at the beginning of the sample sequence. Injection volumes of 1 µL.

Cholesterol Stock Solution Volume Needed to Make the Standard (µL)	Cyclohexane Volume QS into the Volumetric Flask (mL)	Final Concentration of Cholesterol Before Loading (µg/mL)
500	10	50

Comments: _____

Performed By: _____ Date: 06 Apr 21

Reviewed By: _____ Date: _____

Version: 5.0 Effective Date: 03 Apr 2018 10:46:41 GMT Page 1 of 4

Working Copy

Form: QCR-2640-FRM-01

Title: Cholesterol Analysis Data Capture Worksheet

5 Apr 2021 11:22
Balance 14853
SR210126-001
0.0000 g
cholesterol std
100.2 mg

4. Samples Preparation

4.1 Each sample will be prepared in duplicate. The equivalent of 250 µg (100 µL) of cholesterol will be added to a tube containing 5 mL of cyclohexane, giving a final loading concentration of 50 µg/mL. Record volumes in table below. Additional copies of this form can be printed if more than five samples are tested the same day.

CALCULATIONS: $V_{sample} = 250 / C_{sample}$ with V: Volume (µL) and C: Concentration (mg/mL)

Sample	Replicate 1	Replicate 2
ID / Batch Number/Labeled Concentration (mg/mL)	Sample Volume Equivalent to 250 µg Cholesterol (µL)	Sample Volume Equivalent to 250 µg Cholesterol (µL)
10962 | 0000539966	100	100
	05 Apr 21	

4.2 Each sample replicate is:
- Vortex for at least 10 seconds: Record Time (sec): 10 sec
- Shake for 45 min: Record Time (min): 45 min
- Centrifuged for 10 min at 1500 rpms: Record Time (min) and rpms: 10min @ 1500RPM

4.3 A portion of the organic layer will be injected into the GC "as is" in duplicate injections of 1 µL volume.

5. Data Acquisition on Agilent Chemstation

Acquisition Information	File Name	Path Name
Method (Instrument)	CHOLES.M	C:\CHEM32\2\
Method (Shutdown)	SHDOWN.M	SEQUENCE\
Sequence	05APR21.S	05APR21.S
Data	05000001.D - 05000013.D	

Comments: Hydrogen generate Valedation

Performed By: _____ Date: 06 Apr 21

Reviewed By: _____ Date: _____

Form: QCR-2640-FRM-01

Title: Cholesterol Analysis Data Capture Worksheet

6. System Suitability

System Suitability	Criteria	Pass / Fail
Cyclohexane Blank injections	No interfering peak within the retention time window of the cholesterol	Pass
Cholesterol Standard 50 µg/mL:		
Peak Area Response of 6 injections of the standard run before the samples	% CV ≤ 2.0%	fail
% Recovery of the Concentration of the standard run after the samples	95 – 105%	fail

Sigma Cholesterol Standard 50 µg/mL (6 injections)

Injection Number	Ret. Time (min)	Peak Area (pA*s)
1	8.304	64.20137
2	8.304	68.00178
3	8.304	67.33208
4	①~~8.304~~ 8.303	68.72764
5	8.303	69.34881
6	8.303	72.20811
Average	8.304	68.32330
SD	0.001	2.621521
%CV	0.0%	3.836936%

%CV = SD/Average * 100

① Ret time error 06 Apr 21

Cholesterol Std X File No.	Ret. Time (min)	Peak Area (mA280*s)	Calculated Conc. (µg/mL)	%Recovery Concentration
DE 00001153	8.302	72.65558	53.17043	106.34086%

Calculated Conc. (µg/mL) = 50 x pA Std X / pA Std average

% Recovery Concentration = Calculated Conc. Std X / 50 µg/mL * 100

Comments: Validation of Hydrogen generator

06 Apr 21

Performed By: _____ Date: 06 Apr 21

Reviewed By: _____ Date: _____

Data File Z:\GC\OE05APR21\OE05APR21\OE0000002.D
Sample Name: blank

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Acq. Instrument  : 9920-Y-1040                       Location : Vial 1
Injection Date   : 4/5/2021 3:48:00 PM                    Inj :   2
                                                   Inj Volume : 1 µl
Method           : Z:\GC\OE05APR21\OE05APR21\CHOLES.M (Sequence Method)
Last changed     : 4/5/2021 3:25:30 PM by Osagie Evbuoma
Method Info      : Analysis of Cholesterol by GC-FID (QCR-2640)

Sample Info      : Blank Cyclohexane
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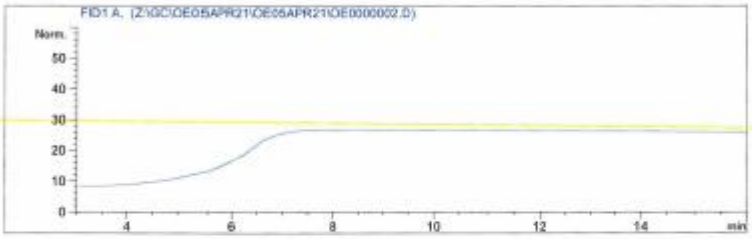


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Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: FID1 A,

Peak RetTime  Type    Width     Area      Area     Name
  #   [min]           [min]   [pA*s]       %
----|-------|------|-------|----------|--------|--------------------
   1   8.860         0.0000    0.00000  0.00000 Cholesterol

Totals :                       0.00000

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

                          *** End of Report ***
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                                                    Inj Volume : 1 µl
Method           : Z:\GC\OE05APR21\OE05APR21\CHOLES.M (Sequence Method)
Last changed     : 4/5/2021 3:25:30 PM by Osagie Evbuoma
Method Info      : Analysis of Cholesterol by GC-FID (QCR-2640)

Sample Info      : Cholesterol Std SR210126-001 50ug/ml
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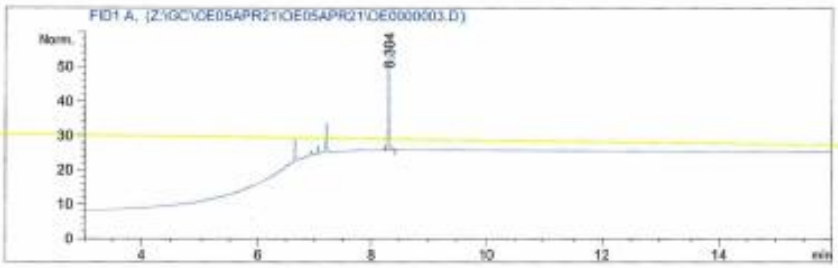


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                          Area Percent Report

Sorted By             :      Signal
Calib. Data Modified  :      2/25/2010 1:04:28 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: FID1 A,

Peak RetTime  Type   Width     Area      Area    Name
  #   [min]          [min]   [pA*s]       %
----|-------|------|-------|----------|--------|--------------------
   1   8.304 BB      0.0341   64.26137 1.0000e2  ?
   2   8.800         0.0000    0.00000  0.00000 Cholesterol

Totals :                      64.26137


1 Warnings or Errors :

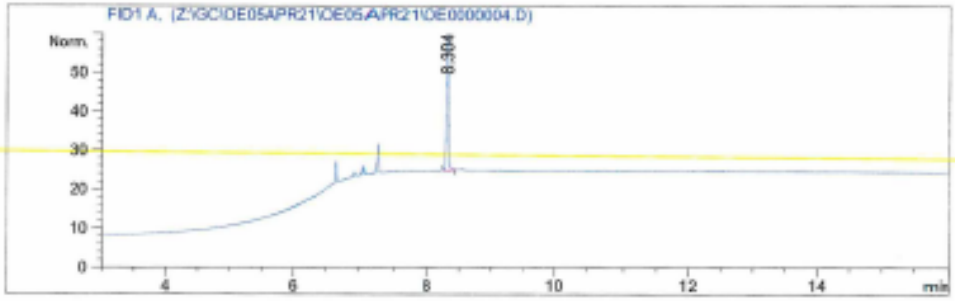
Warning : Calibrated compound(s) not found

                          *** End of Report ***
```

Data File Z:\GC\OE05APR21\OE05APR21\OE0000004.D
Sample Name: std-50ug/ml

```
Acq. Operator    : Osagie Evbuoma                    Seq. Line :   2
Acq. Instrument  : 9920-Y-1040                        Location : Vial 2
Injection Date   : 4/5/2021 4:29:04 PM                    Inj :   2
                                                    Inj Volume : 1 µl
Method           : Z:\GC\OE05APR21\OE05APR21\CHOLES.M (Sequence Method)
Last changed     : 4/5/2021 3:25:30 PM by Osagie Evbuoma
Method Info      : Analysis of Cholesterol by GC-FID (QCR-2640)

Sample Info      : Cholesterol Std SR210126-001 50ug/ml
```




```
                          Area Percent Report

Sorted By             :      Signal
Calib. Data Modified  :      2/25/2010 1:04:28 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: FID1 A,

Peak RetTime  Type   Width     Area      Area     Name
  #   [min]          [min]    [pA*s]       %
----|-------|------|-------|----------|--------|--------------------
   1   8.304 BB      0.0338   68.00178 1.00042  ?
   2   8.800         0.0000    0.00000  0.00000 Cholesterol

Totals :                      68.00178


1 Warnings or Errors :

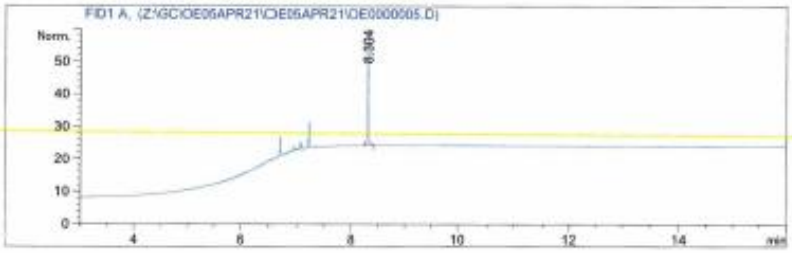
Warning : Calibrated compound(s) not found

                          *** End of Report ***
```

Data File Z:\GC\OE05APR21\OE05APR21\OE0000005.D
Sample Name: std-50ug/ml

```
Acq. Operator    : Osagie Evbuona                    Seq. Line :    2
Acq. Instrument  : 9920-Y-1040                        Location : Vial 2
Injection Date   : 4/5/2021 4:49:32 PM                    Inj :    3
                                                   Inj Volume : 1 µl
Method           : Z:\GC\OE05APR21\OE05APR21\CHOLES.M (Sequence Method)
Last changed     : 4/5/2021 3:25:30 PM by Osagie Evbuona
Method Info      : Analysis of Cholesterol by GC-FID (QCR-2640)

Sample Info      : Cholesterol Std SR210126-001 50ug/ml
```




```
                          Area Percent Report

Sorted By             :      Signal
Calib. Data Modified  :      2/25/2010 1:04:28 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: FID1 A,

Peak RetTime  Type   Width     Area      Area     Name
  #   [min]          [min]    [pA*s]       %
----|-------|------|-------|----------|--------|--------------------
   1   8.304 BB      0.0339   67.33208 1.000e2  ?
   2   8.800         0.0000    0.00000 0.0000O0 Cholesterol

Totals :                      67.33208


1 Warnings or Errors :

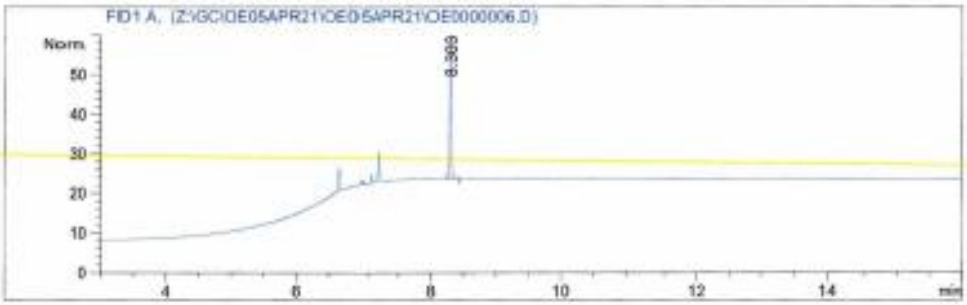
Warning : Calibrated compound(s) not found

                            *** End of Report ***
```

Data File Z:\GC\OE05APR21\OE05APR21\OE0000006.D
Sample Name: std-50ug/ml

Acq. Operator : Osagie Evbuoma Seq. Line : 2
Acq. Instrument : 9920-Y-104 0 Location : Vial 2
Injection Date : 4/5/2021 5:10:02 PM Inj : 4
Inj Volume : 1 µl
Method : Z:\GC\OE05APR21\OE05APR21\CHOLES.M (Sequence Method)
Last changed : 4/5/2021 3:25:30 PM by Osagie Evbuoma
Method Info : Analysis of Cholesterol by GC-FID (QCR-2640)

Sample Info : Cholesterol Std SR210126-001 50ug/ml



Area Percent Report

Sorted By : Signal
Calib. Data Modified : 2/25/2010 1:04:28 PM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	8.303	BB	0.0328	68.72764	1.000e2	?
2	8.800		0.0000	0.00000	0.00000	Cholesterol

Totals : 68.72764

1 Warnings or Errors :

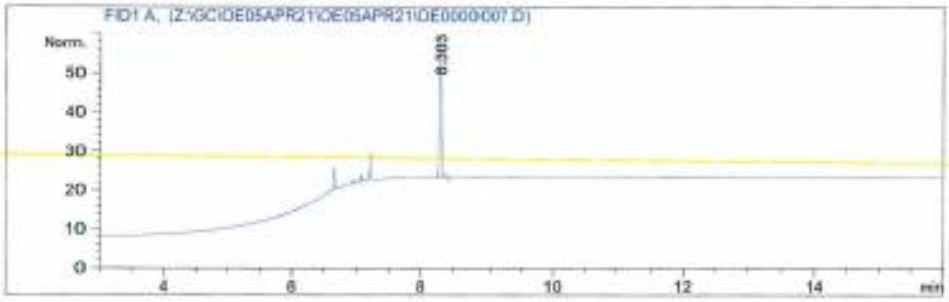
Warning : Calibrated compound(s) not found

*** End of Report ***

Data File Z:\GC\OE05APR21\OE05APR21\OE0000007.D
Sample Name: std-50ug/ml

```
Acq. Operator    : Osagie Evbuoma                    Seq. Line :   2
Acq. Instrument  : 9920-Y-1040                        Location :  Vial 2
Injection Date   : 4/5/2021 5:30:35 PM                    Inj :   5
                                                    Inj Volume : 1 µl
Method           : Z:\GC\OE05APR21\OE05APR21\CHOLES.M (Sequence Method)
Last changed     : 4/5/2021 3:25:30 PM by Osagie Evbuoma
Method Info      : Analysis of Cholesterol by GC-FID (QCR-2640)

Sample Info      : Cholesterol Std SR210126-001 50ug/ml
```




```
                          Area Percent Report

Sorted By             :      Signal
Calib. Data Modified  :      2/25/2010 1:04:28 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: FID1 A,

Peak RetTime  Type    Width     Area      Area     Name
  #   [min]           [min]    [pA*s]       %
----|-------|------|-------|----------|--------|--------------------
   1   8.303 BB      0.0356   69.34881 1.000e2  ?
   2   8.800         0.0000    0.00000 0.00000 Cholesterol

Totals :                      69.34881


1 Warnings or Errors :

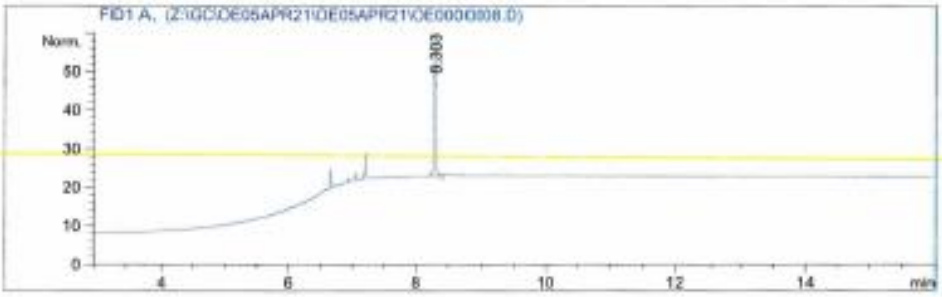
Warning : Calibrated compound(s) not found

                          *** End of Report ***
```

Data File Z:\GC\OE05APR21\OE05APR21\OE000O008.D
Sample Name: std-50ug/ml

```
Acq. Operator    : Osagie Evbuoma                    Seq. Line :   2
Acq. Instrument  : 9920-Y-1040                        Location : Vial 2
Injection Date   : 4/5/2021 5:51:06 PM                    Inj :   6
                                                    Inj Volume : 1 µl
Method           : Z:\GC\OE05APR21\OE05APR21\CHOLES.M (Sequence Method)
Last changed     : 4/5/2021 3:25:30 PM by Osagie Evbuoma
Method Info      : Analysis of Cholesterol by GC-FID (QCR-2640)

Sample Info      : Cholesterol Std SR210126-001 50ug/ml
```




```
                          Area Percent Report

Sorted By             :      Signal
Calib. Data Modified  :      2/25/2010 1:04:28 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: FID1 A,

Peak RetTime  Type    Width     Area      Area    Name
  #   [min]           [min]   [pA*s]       %
----|-------|------|-------|----------|--------|--------------------
   1   8.303 BB      0.0332   72.26811 1.000e2  ?
   2   8.800         0.0000    0.00000  0.00000 Cholesterol

Totals :                      72.26811


1 Warnings or Errors :

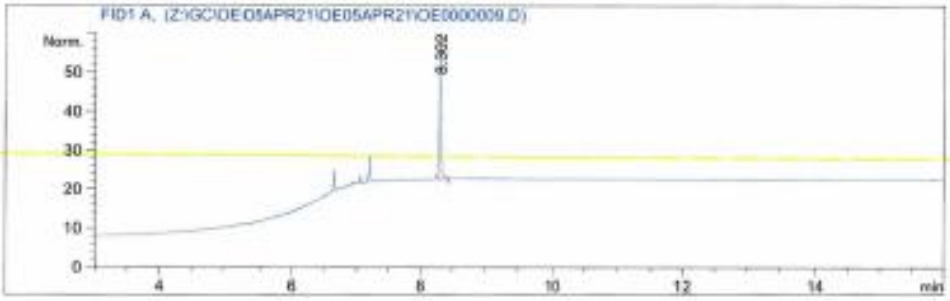
Warning : Calibrated compound(s) not found

                          *** End of Report ***
```

Data File Z:\GC\OE05APR21\OE05APR21\OE0000009.D
Sample Name: 0001539966-1 Val

```
Acq. Operator    : Osagie Evbuoma                    Seq. Line :    3
Acq. Instrument  : 9920-Y-1040                        Location : Vial 3
Injection Date   : 4/5/2021 6:11:33 PM                    Inj :    1
                                                    Inj Volume : 1 µl
Method           : Z:\GC\OE05APR21\OE05APR21\CHOLES.M (Sequence Method)
Last changed     : 4/5/2021 3:25:30 PM by Osagie Evbuoma
Method Info      : Analysis of Cholesterol by GC-FID (QCR-2640)

Sample Info      : 10962/0001539966-1
```




```
                          Area Percent Report

Sorted By             :      Signal
Calib. Data Modified  :      2/25/2010 1:04:28 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: FID1 A,

Peak RetTime  Type    Width     Area      Area    Name
  #   [min]           [min]   [pA*s]       %
----|-------|------|-------|----------|--------|--------------------
   1   8.302 BB      0.0353   76.98200 1.0000e2  ?
   2   8.800         0.0000    0.00000  0.00000 Cholesterol

Totals :                      76.98200


1 Warnings or Errors :

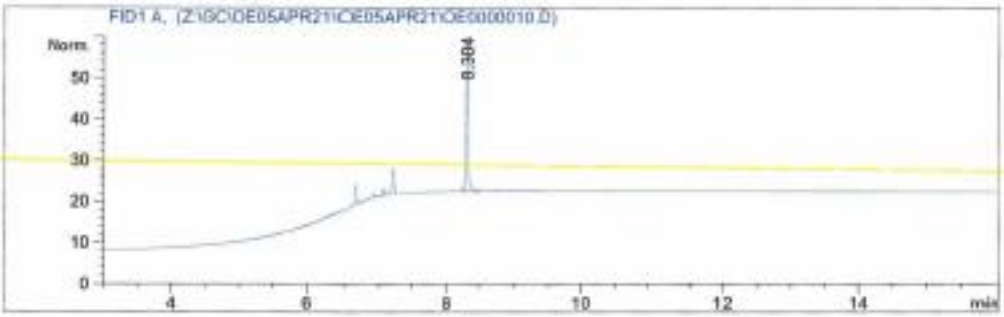
Warning : Calibrated compound(s) not found

                          *** End of Report ***
```

Data File Z:\GC\OE05APR21\OE05APR21\OE0000010.D
Sample Name: 0001539966-1 Val

```
================================================================================
Acq. Operator    : Osagie Evbuoma                    Seq. Line :   3
Acq. Instrument  : 9920-Y-1040                        Location : Vial 3
Injection Date   : 4/5/2021 6:32:07 PM                    Inj :   2
                                                    Inj Volume : 1 µl
Method           : Z:\GC\OE05APR21\OE05APR21\CHOLES.M (Sequence Method)
Last changed     : 4/5/2021 3:25:30 PM by Osagie Evbuoma
Method Info      : Analysis of Cholesterol by GC-FID (QCR-2640)

Sample Info      : 10962/0O01539966-1
```




```
================================================================================
                          Area Percent Report
================================================================================

Sorted By             :      Signal
Calib. Data Modified  :      2/25/2010 1:04:28 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: FID1 A,

Peak RetTime  Type   Width     Area      Area     Name
  #   [min]          [min]   [pA*s]       %
----|-------|------|-------|----------|--------|--------------------
   1   8.304 BB      0.0360   77.66113 1.0002   ?
   2   8.800         0.0000    0.00000  0.0000 Cholesterol

Totals :                      77.66113


1 Warnings or Errors :

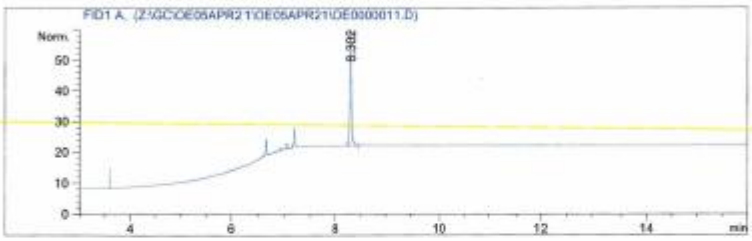
Warning : Calibrated compound(s) not found

================================================================================
                          *** End of Report ***
```

Data File Z:\GC\OE05APR21\OE05APR21\OE0000011.D
Sample Name: 0001539966-2 Val

Acq. Operator	: Osagie Evbuoma	Seq. Line :	4
Acq. Instrument	: 9920-Y-1040	Location :	Vial 4
Injection Date	: 4/5/2021 6:52:35 PM	Inj :	1
		Inj Volume :	1 µl
Method	: Z:\GC\OE05APR21\OE05APR21\CHOLES.M (Sequence Method)		
Last changed	: 4/5/2021 3:25:30 PM by Osagie Evbuoma		
Method Info	: Analysis of Cholesterol by GC-FID (QCR-2640)		

Sample Info : 10962/0001539966-2



Area Percent Report

Sorted By : Signal
Calib. Data Modified : 2/25/2010 1:04:28 PM
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	8.362	BB	0.0364	77.10297	1.0000e2	?
2	8.800		0.0000	0.00000	0.00000	Cholesterol

Totals : 77.10297

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

*** End of Report ***